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Zebrafish (*Danio rerio*) as a model for the study of aging and exercise: Physical ability and trainability decrease with age

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ABSTRACT

A rapidly aging global population has motivated the development and use of models for human aging. Studies on aging have shown parallels between zebrafish and humans at the internal organization level; however, few parallels have been studied at the whole-organism level. Furthermore, the effectiveness of exercise as a method to mitigate the effects of aging has not been studied in zebrafish. We investigated the effects of aging and intermittent exercise on swimming performance, kinematics and behavior. Young, middle-aged and old zebrafish (20–29, 36–48 and 60–71% of average lifespan, respectively) were exercised to exhaustion in endurance and sprint swimming tests once a week for four weeks. Both endurance and sprint performance decreased with increased age. Swimming performance improved with exercise training in young and middle-aged zebrafish, but not in old zebrafish. Tail-beat amplitude, which is akin to stride length in humans, increased for all age groups with training. Zebrafish turning frequency, which is an indicator of routine activity, decreased with age but showed no change with exercise. In sum, our results show that zebrafish exhibit a decline in whole-organism performance and trainability with age. These findings closely resemble the senescence-related declines in physical ability experienced by humans and mammalian aging models and therefore support the use of zebrafish as a model for human exercise and aging.

1. Introduction

In the next 50 years, the proportion of the world's population aged 60 years or older will double (Lutz et al., 2008) and this has motivated the study of the mechanisms, effects and mitigation of aging. In biogerontology, a wide array of model organisms is available to allow for a comparative and integrative study of aging (Holmes and Kristan, 2008). One promising model organism currently used is the zebrafish (*Danio rerio*).

Zebrafish have many advantages as a model system for aging research. They experience senescence, have an evolutionarily conserved genome and are genetically manipulatable (Gerhard, 2003; Lieschke and Currie, 2007). Furthermore, as many zebrafish studies continue to point out, zebrafish are inexpensive and highly fecund. Together, these characteristics suggest that zebrafish could be strong models for large-scale, high-throughput pharmacological and genetic screens for the study of aging and aging intervention (Kishi, 2011; Sasaki and Kishi, 2013; Zon and Peterson, 2005). However, the utility of zebrafish as an aging model has been questioned because of their strong regenerative abilities and possible indeterminate growth (Keller and Murtha, 2004). Yet, since zebrafish regenerative ability declines with age, and the likelihood of physiological damage increases, gradual senescence still occurs (Kishi, 2004, 2011).

Aging can be defined relative to the scope of interest. For our study, we define aging as the accumulation of deleterious changes over multiple levels of biological organization that results in a decrease in whole-organism functionality with time (as in Weinert and Timiras, 2003). Many sub-organismal similarities have been identified in aging humans and zebrafish. For example, both aging humans and zebrafish experience an accumulation of oxidized proteins, a decline in mitochondrial functionality, and a degeneration of skeletal muscle (Doherty, 2003; Gerhard and Cheng, 2002; Kishi et al., 2003; Short et al., 2005; Visser et al., 2005). At present, few studies have investigated whether aging affects whole-organism performance in zebrafish in a similar manner as humans and mammalian aging models.

The primary objective of our study was to determine the utility of zebrafish as a model for human aging. A secondary objective was to determine if zebrafish could demonstrate the utility of exercise as an intervention strategy. Thus, we investigated the independent and interactive effects of aging and intermittent exercise on commonly used fish exercise protocols: swimming performance. Swimming performance

Abbreviations: TBA, tail-beat amplitude; TBF, tail-beat frequency; fps, frames per second; U_{crit}, maximum endurance $swimming speed; U_{max}, maximum sprint swimming speed; BL, standard body length; f , turning frequency.

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is an endpoint that requires the functioning of many systems from the molecular to organ level; therefore, it can capture the consequences of aging. We examined sprint and endurance swimming abilities and some underlying kinematics. We also examined zebrafish turning frequency, as this can be an indicator of the ability to perform routine behaviors (Plaut, 2001). We hypothesized that if zebrafish experience aging in a manner similar to humans, then zebrafish swimming performance would decline with age.

Exercise is currently used as an aging intervention strategy for elderly humans to improve their muscles' ability to respond to the stress of physical activity (Koopman and Van Loon, 2009). With our model organism, exercise can be achieved by forcing them to swim against increased water velocities (Kieffer, 2010; Plaut, 2001; Young and Cech, 1993). We used intermittent exercise, consisting of weekly bouts of exhaustive swimming, to examine the effect of aging on swimming performance trainability. A study on juvenile largemouth bass (*Micropterus salmoides*) used fingerlings used a similar protocol and found improvements in swimming ability (Macleod, 1967). As exercise training tends to improve the overall condition of animals (LeMoine et al., 2010), we expected training to increase zebrafish swimming ability. However, since regenerative ability declines and physiological damage increases with age (Kishi, 2004, 2011), we expected that trainability would be diminished in older zebrafish.

2. Materials and methods

2.1. Animal care and ethics

At the start of the study, AB strain zebrafish (*D. rerio*) were divided into three age groups (Fig. 1b): young (8–12 months; n = 7, mass = 0.42 ± 0.04 g, body length = 28.4 ± 0.75 mm), middle-aged (15–20 months; n = 8, mass = 0.62 ± 0.09 g, body length = 31.6 ± 1.17 mm) and old (25–30 months; n = 7, mass = 0.58 ± 0.08 g, body length = 30.6 ± 0.95 mm). The ages of young, middle-aged and old zebrafish correspond to 20–29%, 36–48%, and 60–71% of the average life lifespan (40 months), respectively. These age groups are similar to those in previous studies that demonstrated detrimental sub-organismal effects of aging in zebrafish (da Rosa et al., 2010; Kishi et al., 2003; Murtha and Keller, 2003). The number of zebrafish of each gender was split evenly at the start of the study. For animal care, one male and one female zebrafish of the same age group were held in one quarter-liter flow-through tanks. Zebrafish were fed with brine shrimp twice daily to satiation and were kept on a 14/10 light/dark cycle photoperiod at 28 °C. Zebrafish housing and experimental protocols followed standard care procedures (Westerfield, 1994) and guidelines set by the University of Alberta Ethics Policy and Canadian Council on Animal Care in Science (Animal use protocol: 22). During the course of the study, 5 of the 23 zebrafish were removed due to illness, injury or death (young = 1, middle-aged = 1 and old = 3). Once a zebrafish missed a single test it was excluded for the remainder of the study.

2.2. Swimming chamber

Fish were placed in a 10 L swim tunnel with an adjustable flow that forced fish to swim to maintain their position (Fig. 1a; Loligo Systems, DE). The test section was 30 cm long, 10 cm wide and 10 cm high, which was large enough to allow zebrafish to perform various swimming gaits. Water temperature was maintained at acclimation temperature (28 °C). Zebrafish were tested under infrared light to reduce any visual disturbances that could affect swimming. An infrared camera (recorded at 30 frames per second (fps); Matco, QE) and high-speed cameras (recorded at 60 fps, Gopro Hero2; 240 fps, Casio EX-ZR10) were mounted above the swim tunnel to record the swimming tests.

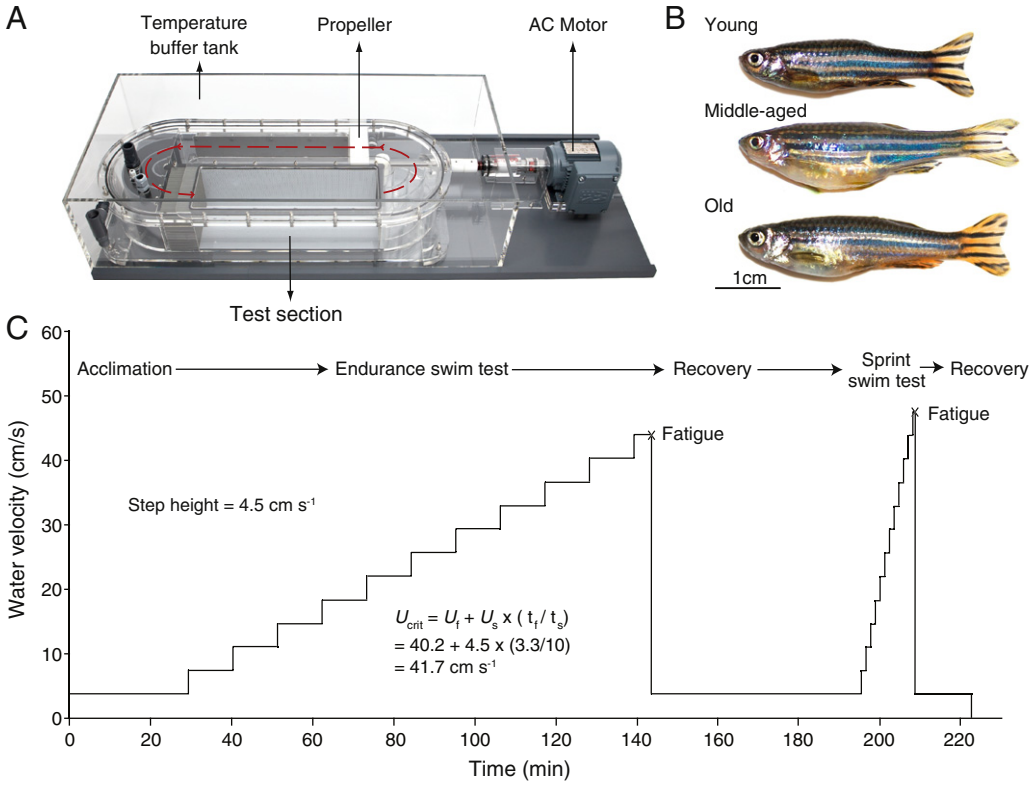

Fig. 1. The swim tunnel (A), representative zebrafish (B), and the protocol used in exercise training and swimming assessments (C). Zebrafish were placed individually in the test section and subjected to the exercise procedure shown, once per week for four weeks. Water velocity was adjusted using an electronic controller and an AC motor driving a propeller. The red broken line indicates flow direction. In the formula, U_f is the water velocity of the last completed step, U_s is the increase in water velocity of each step, t_f is the length of time the fish swam before fatigue, and t_s is the duration of a step. Photo courtesy of LoligoSystems.com.

2.3. Swimming performance protocol

Zebrafish were given an endurance-swimming test (U_{crit}) followed by a sprint test (U_{max}) once weekly for four consecutive weeks. The order that zebrafish were selected for testing was randomized in the first week of the study. This order was maintained throughout the study so that fish were tested at exactly one-week intervals. Zebrafish were placed into the swim tunnel and acclimated at a low flow speed (4.5 cm s^{-1}) for 30 min (Fig. 1c). After this acclimation, fish were given the U_{crit} test, during which water velocity was increased gradually by 4.5 cm s^{-1} in 10 min steps until zebrafish fatigued. Fatigue was operationally defined as the point at which a fish stopped swimming and was forced against the rear of the test section for greater than 5 s. The U_{crit} test is an indicator of aerobic swimming ability (Brett, 1964; Tierney, 2011). Following fatigue, fish were allowed to recover for 45 min at low flow speed (4.5 cm s^{-1}). The recovery period was defined as a period of swimming slow and long enough to allow a fish to achieve 100% of its initial performance in a subsequent test (Jain et al., 1998; Peake et al., 1997). After the recovery period, fish were given a U_{max} test, during which water velocity was increased rapidly by 4.5 cm s^{-1} in 1 min steps. This test is used as an indicator of anaerobic swimming ability (Tierney, 2011). After the zebrafish fatigued in the U_{max} test, they were recovered at the low flow speed (4.5 cm s^{-1}) for 15 min. Fish were then anesthetized in 0.65 mM Tris-buffered, tricaine methanesulfonate (Syndel, BC, Canada), then weighed (±0.01 g) and measured for standard body length (BL, measured from the tip of the mouth to the end of the axial musculature; ±0.1 mm). Both U_{crit} and U_{max} were calculated using the Brett equation (1964; Fig. 1c): $U_{crit/max} = U_f + U_s \times (t_f / t_s)$, where U_f is the water velocity of the last completed step before fatigue, U_s is the increase in water velocity each step, t_f is the length of time the fish swum before fatigue in the final step, and t_s is the duration of a step.

During the swimming performance tests, some fish were able to bite onto the front gate of the test section and briefly stop swimming. Videos were analyzed for biting duration, and this was subtracted from swimming times. For a biting attempt to be included it had to be over one second long as fish did not stop swimming within the first second of holding. Biting was corrected in 13 of the 82 U_{crit} tests run (i.e. 16% of trials; young = 6, middle-aged = 5, old = 2). On average, biting was first observed in the 22.4 cm s^{-1} step. The average bite duration was 3.4 ± 0.2 s, and the total bite duration per trial was 3:07 ± 0:58 min. While some recovery may have occurred during the biting periods it was likely marginal due to the short duration of the bites and because biting would necessitate a closed mouth, which would have greatly reduced gill ventilation and therefore oxygen uptake. We recorded excessive biting (>10 min) in one young fish during week 4 and felt that recovery may have occurred so the test was omitted.

2.4. Swimming kinematics and behavior

The frequency and amplitude of tail beats (TBF and TBA, respectively) were measured at an intermediate speed (22.4 cm s^{-1}) in weeks one and four using frame-by-frame analysis of high-speed video (QuickTime, Apple, CA, USA) in a similar manner to Marit and Weber (2011). The intermediate speed was chosen because it was high enough to force zebrafish to maintain steady swimming (with low turning frequency), while not inducing fatigue in most fish. The number of tail beats that occurred in 1 s was counted three separate times and the counts were averaged to give the TBF. A single tail beat was considered as a movement of the tail from right to left and back. For each tail beat counted, the amplitude was taken by summing the right and left lateral deflections from the midline of the body. Measurements were made using a pixel ruler (Free Ruler v1.7, Pascal, NY, USA), and the known width of the test section (10 cm) for scale. The TBA was analyzed and reported as a percentage of BL to account for any influence of fish size on TBA (Bainbridge, 1958).

To assess turning behavior, we counted the number of times zebrafish turned per min at each step from recorded video. A turn was considered as a change in head orientation of greater than 90° away from the direction of water flow. The angle was taken to be greater than 90° if the midpoint of a fish's head was behind the midpoint of its body, which was determined using a pixel ruler parallel with the direction of water flow. The number of turns per min was counted three separate times during each velocity step until the fish stopped turning. The turning frequency (f_T) at each velocity step was calculated as the average of the three measurements. A second, naive observer counted turning in three U_{crit} tests to determine the inter-rater reliability for this method. The intraclass correlation coefficient (ICC) was 0.977 (95% confidence interval (C.I.): 0.955–0.988) for 58 f_T measurements taken by each observer, suggesting that the values from the first observer were reliable.

2.5. Data analysis

We used a three-factor, repeated measures general linear model to determine the influence of gender, age and exercise on swimming performance. There were no differences in swimming ability between the sexes ($F_{1,11} = 0.026$ P = 0.875), which agrees with findings of a previous study (Marit and Weber, 2011). As we found no gender differences and gender distribution was equal across groups, male and female data were pooled in subsequent analyses. We performed two-way repeated measures analyses of variance (RM-ANOVA) to detect whether exercise and aging affect swimming performance and kinematics. For swimming performance, this analysis was conducted on residuals from linear-regressions between body-length and U_{crit} or U_{max} to remove the influence of body-size on swimming performance. The Student–Newman–Keuls multiple comparisons adjustment was performed for pairwise comparisons between all groups. The effects of age, exercise and water velocity on turning frequency were modeled using multiple linear regression analysis. The inter-rater reliability for measurements of turning behavior was calculated as an ICC with a 95% C.I. using a two-way mixed effects model testing absolute agreement. The statistical analysis and data presentation was completed using SPSS 20 (IBM, IL, USA) and SigmaPlot 11 (Systat Software, CA, USA).

3. Results

3.1. Swimming performance

Zebrafish swimming performance decreased with age (U_{crit}: $F_{2,81} = 17.832$, P < 0.001; U_{max}: $F_{2,81} = 23.851$, P < 0.001; Figs. 2 and 3). Overall, all three age groups differed from each other (RM-ANOVA; see Table 1 for pairwise comparisons). Relative to their body lengths, young zebrafish had higher endurance and sprint swim speeds than middle-aged and old zebrafish (middle-aged: U_{crit}: +13%, P = 0.03; U_{max}: +11%, P = 0.02; old: U_{crit}: +45%, P < 0.01; U_{max}: +42%, P < 0.01; Figs. 2 and 3; Table 1). Middle-aged zebrafish reached greater swim speeds than old zebrafish (U_{crit}: +29%, P < 0.01; U_{max}: +28%, P < 0.01; Figs. 2 and 3; Table 1).

Swimming performance improved with exercise (U_{crit}: $F_{3,81} = 4.901$, P = 0.005; U_{max}: $F_{3,81} = 5.405$, P = 0.003; Figs. 2 and 3). The increase from weeks one to four was significant in young and middle-aged zebrafish, but not in old (young: U_{crit}: +23%, P = 0.05; U_{max}: +23%, P = 0.02; middle-aged: U_{crit}: +23%, P = 0.05; U_{max}: +25%, P = 0.02; old: U_{crit}: +10%, P = 0.81; U_{max}: +5%, P = 0.65; Fig. 2; Table 1). There was no interaction between exercise training and age (U_{crit}: $F_{6,81} = 0.537$, P = 0.78; U_{max}: $F_{6,81} = 1.105$, P = 0.37).

3.2. Swimming kinematics

There was no change in TBF with either age or training (age: $F_{2,27} = 0.0711$, P = 0.93; training: $F_{1,27} = 1.564$, P = 0.24). The TBA

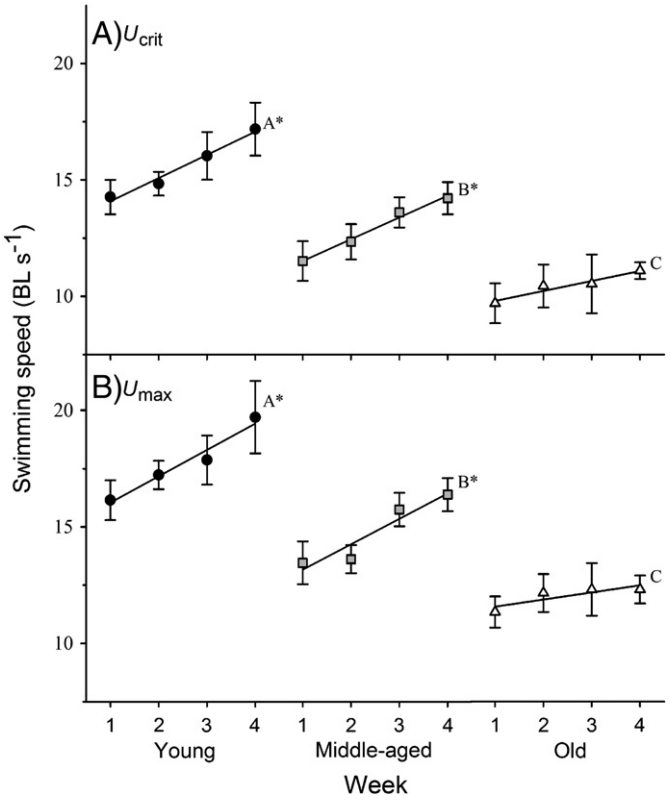

Fig. 2. Zebrafish swimming performance across three age groups over four weeks of intermittent exercise. Dissimilar letters indicate overall differences between age groups ($P \leq 0.05$). Asterisks represent differences between weeks one and four of exercise training ($P \leq 0.05$). Data are presented as mean ± SEM.

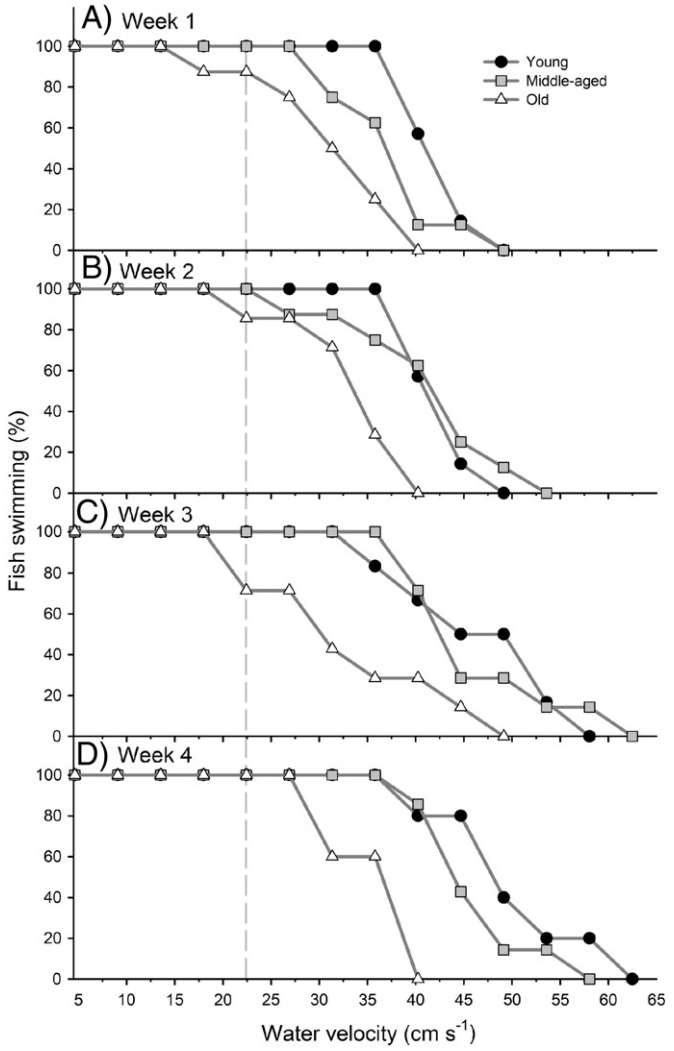

Fig. 3. Zebrafish fatigue curves across three age groups over four weeks of intermittent exercise. Data are shown as the percentage of fish in each age group that were able to continue swimming at the water velocity steps. The broken vertical line indicates the velocity at which tail-beat frequency and amplitude were measured (22.4 cm/s).

increased with exercise ($F_{1,27} = 19.710$, $P = 0.01$; Fig. 4) and did not differ between age groups ($F_{2,27} = 0.729$, $P = 0.50$). All age groups increased TBA from weeks one to four (young: 29.0 ± 1.2 to 37.4 ± 2.2% BL, $P = 0.02$; middle-aged: 29.3 ± 1.2 to 33.8 ± 2.7% BL, $P = 0.07$; old: 30.2 ± 1.3 to 38.0 ± 3.1% BL, $P = 0.02$; Fig. 4).

3.3. Swimming behavior

The f_T decreased with age and did not change with exercise (Table 2; Fig. 5). As expected, all age groups decreased f_T as water velocity increased. A multiple linear regression model including water velocity, age and exercise status accounted for 44% of f_T variability ($F_{4,149} = 28.691$, $P < 0.01$; Table 2).

4. Discussion

Previous studies have shown that humans and other mammals exhibit a decline in whole-organism physical performance with age (Baker and Tang, 2010; Carter et al., 2002; Tanaka and Seals, 2003); our study demonstrates that zebrafish experience a similar decline. This decline has not previously been investigated in fish. We also determined that swimming performance-associated kinematics change with exercise and that routine activity during exercise decreases with age. We suggest that zebrafish swimming performance and behavior are endpoints that can be used to examine the effects of aging and training.

4.1. Age-dependent changes in physical performance

Maximal running speeds decline with age in humans, mice and rats (Evans et al., 1995; Ingram, 1988; Podolin et al., 1994; Tanaka and Higuchi, 1998; Fig. 6). We found that swimming ability also declined as zebrafish aged from young to middle-aged to old (Fig. 6). Specifically, zebrafish exhibited a 6.2% decline in maximal performance for every 10% progression of average lifespan over the tested age range. This decline is very similar to humans and rats, which exhibited a 7.5 and 5.3% decline in running performance for every 10% progression in lifespan, respectively (Evans et al., 1995; Podolin et al., 1994). In fish and mammals the natural modes of locomotion are swimming and running, respectively. Performance tests in both groups of animals can be designed to have similar aerobic and anaerobic demands, be of similar durations and involve the use of variable gaits (as speed increases, both can modify the frequency and amplitude of strides). The comparison of performance measured using different modes of locomotion is further supported by a human study on Masters athletes that found maximal endurance performance in swimming, rowing, cycling and running decreased with age at nearly identical rates (Baker and Tang, 2010).

Numerous factors likely contribute to declines in physical ability in vertebrates, as both running and swimming require the integration of many physiological systems. In aging humans, reduced physical ability is partially attributed to sarcopenia, i.e. the loss of muscle mass and associated strength (Doherty, 2003; Lushaj et al., 2008). In old zebrafish, an increased frequency of severe spinal curvature has been suggested to be caused by abnormal muscle structure, indicating sarcopenia (Gerhard and Cheng, 2002). In our study, changes in both body length

Table 1
Swimming performance of young, middle age and old fish over four weeks of intermittent exercise.

Week	Young					Middle-aged					Old				
	N	Mass (g)	Length (mm)	U_{crit} (cm s^{-1})	U_{max} (cm s^{-1})	N	Mass (g)	Length (mm)	U_{crit} (cm s^{-1})	U_{max} (cm s^{-1})	N	Mass (g)	Length (mm)	U_{crit} (cm s^{-1})	U_{max} (cm s^{-1})
1	7	0.42 ± 0.04	28 ± 1	40.2 ± 1.2^{Aa}	45.5 ± 1.3^{Aa}	8	0.62 ± 0.09	32 ± 1	35.9 ± 1.9^{Ab}	42.0 ± 1.9^{Aa}	8	0.58 ± 0.08	31 ± 1	29.5 ± 2.5^{c}	34.6 ± 2.0^{b}
2	7	0.41 ± 0.04	28 ± 1	41.4 ± 1.0^{a}	48.0 ± 1.1^{a}	8	0.60 ± 0.08	32 ± 1	39.2 ± 3.0^{a}	43.1 ± 2.6^{Ab}	7	0.56 ± 0.01	30 ± 1	31.4 ± 2.5^{b}	36.7 ± 2.4^{b}
3	6	0.39 ± 0.04	28 ± 1	44.5 ± 3.4^{a}	49.4 ± 3.3^{a}	7	0.63 ± 0.08	33 ± 1	44.1 ± 3.0^{a}	50.9 ± 2.9^{Ba}	7	0.53 ± 0.09	30 ± 1	31.3 ± 3.8^{b}	36.5 ± 3.4^{b}
4	5	0.39 ± 0.06	29 ± 1	48.1 ± 3.3^{Ba}	55.1 ± 4.4^{Ba}	7	0.60 ± 0.08	32 ± 1	44.7 ± 2.2^{Ba}	51.5 ± 2.4^{Ba}	5	0.56 ± 0.11	31 ± 1	34.3 ± 2.5^{b}	38.1 ± 3.3^{b}

Dissimilar capital letters indicate significant differences within columns ($\alpha = 0.05$).
Dissimilar lowercase letters indicate significant differences between ages for U_{crit} or U_{max} ($\alpha = 0.05$).

and mass suggest that sarcopenia may be occurring in old zebrafish, as they tended to be lighter and shorter than middle-aged fish. Therefore, the observed decrease in zebrafish swimming performance with age could partially be a result of reduced muscle mass and functionality. It may be important to note that biting (of the front gate) occurred more in young and middle aged zebrafish than old zebrafish, and even though biting was of brief durations and observed only in a few trials (16%), it is possible that it permitted some small recovery advantage that allowed fish to swim longer.

4.2. Improved physical ability with exercise

Exercise performance improves with training in humans, other mammals and fish. After interval or endurance training, humans improved in cycling performance trials by decreasing completion times of 2 and 30 km distance tests by 3.5–4.1 and 7.5–10.1%, respectively (Gibala et al., 2006). In mice and rats, endurance training improved their exercise performance in running to exhaustion tests by nearly 85 and 233%, respectively (Davidson et al., 2006; Dolinsky et al., 2012). In our study, swimming performance improved over the four-week training period in young and middle-aged zebrafish by 23–25%. This improvement in swimming performance is corroborated by other studies. For example, other exercise-conditioned fish species swam 10–40% faster than untrained fish in swimming performance tests (Farrell et al., 1990; Ward and Hilwig, 2003; Young and Cech, 1993). Similar to our study, Macleod (1967) found that intermittent exercise training of largemouth bass (*M. salmoides*) fingerlings improved their maximum swimming speed by an average of 63–69%. The improvements in performance across species argue that vertebrate models, regardless of being endo- or ecto-therms, show the benefits of exercise in mitigating the effects of aging.

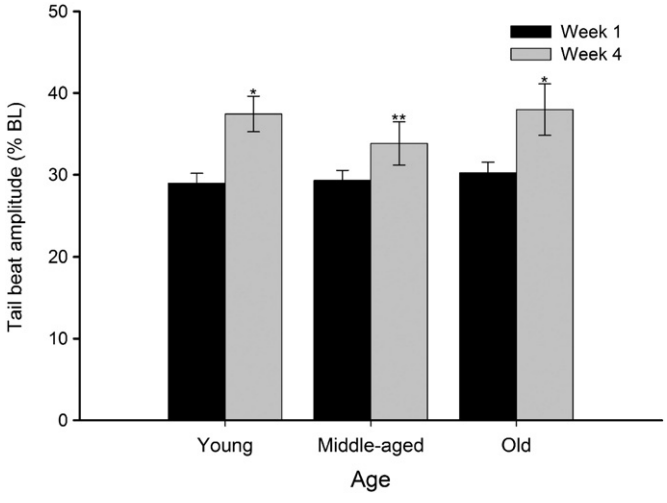

Fig. 4. Zebrafish tail beat amplitude across three age groups between weeks one (black) and four (gray) of intermittent exercise training. Asterisks * and ** indicate $P < 0.05$ and $P = 0.07$, respectively, for comparisons between weeks one and four. Data are presented as mean ± SEM.

In contrast to young and middle-aged zebrafish, old zebrafish only improved their swimming performance by 5–10%. The lack of statistically significant improvement with training suggests that aging may reduce the physiological plasticity responsible for the improvement of whole-organism performance. In humans, a decline in exercise-induced plasticity occurs with age. For example, older males had lower anabolic signaling and muscle protein synthesis rates following resistance exercise when compared to younger males (Kumar et al., 2009). However, in a study using a six-month endurance and resistance exercise program, elderly women improved their maximum workload by 31% (Strasser et al., 2009). Thus, the minor improvements observed in aging zebrafish suggest that, while exercise-induced plasticity may be reduced, it may be possible to achieve significant improvements in physical performance with a longer and more intense exercise regime.

The ability of aging individuals to exercise may have also limited their improvement. Old zebrafish failed earlier in exercise tests than younger zebrafish, meaning that their exercise bouts were shorter and not as rigorous. This decrease in duration and intensity of exercise could also account for part of the age-dependent effect of trainability. Fatouros et al. (2005) found that a more pronounced improvement in elderly humans' fitness was obtained with higher intensity training protocols. Future zebrafish studies could investigate the relationship between training intensity and duration, physiological plasticity, and whole-organism performance.

4.3. Change in locomotor kinematics

TBA and TBF were measured in weeks one and four in order to reveal any effects of exercise and age on swimming kinematics. TBA increased with exercise for all age groups, whereas TBF did not change with age or training. An increase in TBA without a change in TBF corresponds to an increased propulsive force, which would allow fish to better maintain their position in a water flow current (Bainbridge, 1958). Unlike young and middle-aged zebrafish, the increase in TBA for old zebrafish did not correspond to an increase in swimming performance, which suggests that the ability to modulate locomotor kinematics (gait) was not a critical factor limiting the trainability of aged zebrafish.

In humans, gait characteristics vary with age and exercise. Various exercise programs increase step length in elderly humans, which is similar to the exercise-induced increase in TBA observed in zebrafish

Table 2
Multiple-linear regression model of turning frequency. Predictor variables include water velocity, age and exercise status (week 1 vs. 4).

Predictor	Coefficient	Standard Error	*t*	P
Constant	17.665	1.355	13.037	<0.001
Log(U(cm s^{-1}))	−11.42	1.147	−9.96	<0.001
Young	Reference term	–	–	–
Middle-age	−1.354	0.671	−2.019	0.045
Old	−3.609	0.712	−5.072	<0.001
Trained	−0.568	0.572	0.992	0.323

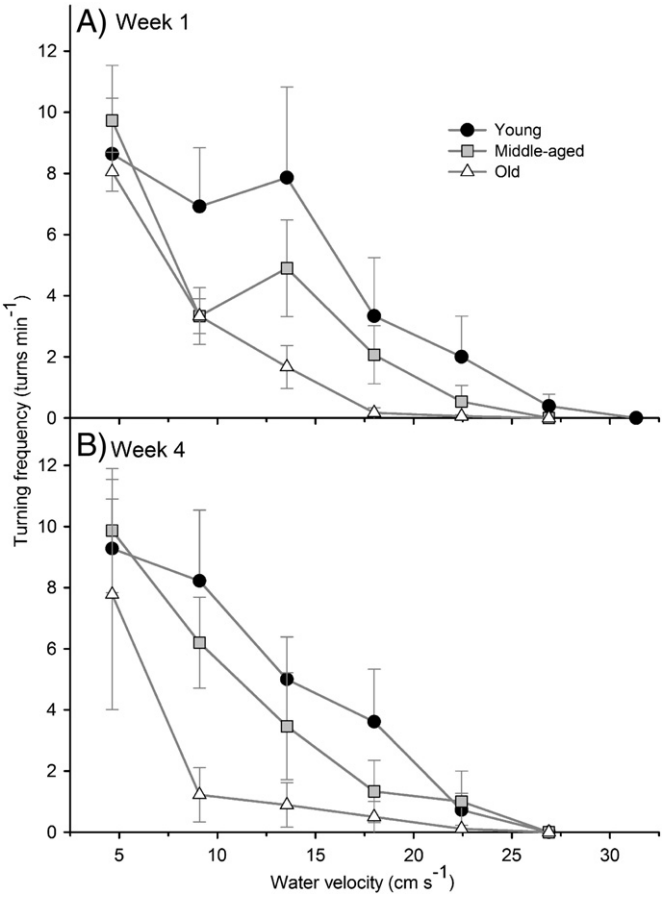

Fig. 5. Zebrafish turning frequency across three age groups for weeks one and four of intermittent exercise training. Data are presented as mean ± SEM. For regression statistics, see Table 2.

(Iwamoto et al., 2004; Kawanabe et al., 2007; Lord et al., 1996). In the same studies, both step length and step frequency (similar to TBF) decreased with age and were accompanied by decreased walking speed. We did not find equivalent age-dependent changes in zebrafish. However, changes in locomotor kinematics may not be apparent until later in the zebrafish lifespan, as subjects in human gait studies tend to be older relative to their average lifespan than the old zebrafish used in this study.

4.4. Decreased scope for routine activity with age

Turning frequency (f_T) is a measurement of routine activity during exercise (Plaut, 2001). In a water flow current, zebrafish maintain their position by swimming upstream, but often routinely turn downstream causing them to drift (Plaut, 2000). All zebrafish decreased f_T as water speed increased, similar to findings by Plaut (2000). This decrease was expected, as effort in maintaining position at higher water flow speeds increases. f_T also decreased with age, indicating an age-dependent decline in either motivation or ability to carry out routine activities while exercising. Decreased locomotor activity has been reported before in aging zebrafish; however, it was not apparent until three years of age (Zhdanova et al., 2008). In our study, the decrease in activity was evident by 15–20 months. As older zebrafish have a lower maximum swimming ability, they likely have to exert a greater portion of their maximal effort to maintain their position in flow at any given speed. This increase in relative effort means that older fish have a lower scope for routine activity and if they were to turn at the higher speeds observed in young fish there would be a greater likelihood that they would not be able to recover their position.

Old mice and elderly humans experience similar declines in routine behaviors and spontaneous activity (Doherty, 2003; Valentinuzzi et al., 1997). Navarro et al. (2004) found that exploratory behavior and whole-organism performance in mice decreased with age, but increased with moderate exercise. These findings are consistent with our observed reductions in routine activity and swimming ability with age

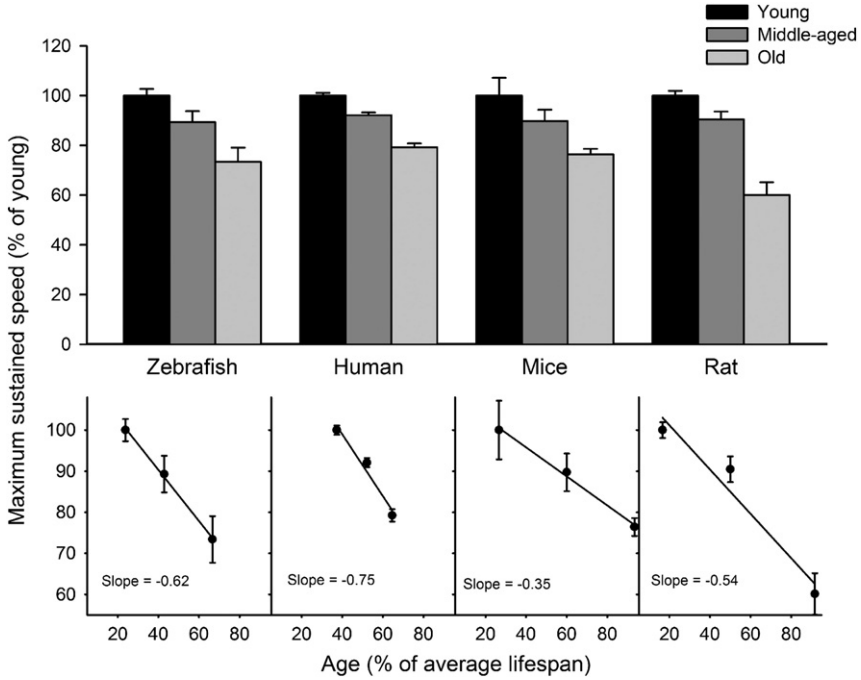

Fig. 6. A comparison of changes in peak performance across age groups in zebrafish, humans, mice and rats. The data are expressed as a percentage of "young" performance and presented as mean ± SEM. Data for human, mice and rats are calculated from Evans et al. (1995) Ingram (1988), and Podolin et al. (1994), respectively.

and the improved performance with exercise; however, we did not find that exercise increased routine activity in any age group. This indicates that in zebrafish, routine activity is independent of maximum energetic-based performance.

4.5. Reproducible whole-organism performance metrics

We found that zebrafish swimming performance and behavior are reproducible whole-organism endpoints. Young zebrafish had a mean U_{crit} of 15.4 BL/S, which is nearly identical to other similarly aged zebrafish from another study that had a mean U_{crit} of 15.5 BL/S (Plaut, 2000). Furthermore, the differences in swimming performance among age groups were reproducible over the four weeks of training, indicating consistent measurement of age-specific swimming ability. f_T at the onset of current was 9.3 turns min^{-1} and 9.8 turns min^{-1} for our young and middle-aged zebrafish, respectively. Plaut (2000) determined average f_T of young zebrafish to be 10.1 turns min^{-1}, which is similar to our values. Together, these findings suggest that swimming performance and turning frequency are reliable, reproducible metrics for zebrafish whole-organism performance. For this reason, zebrafish swimming performance and behavior can be used as whole-organism endpoints in the study of aging and exercise.

4.6. The future use of zebrafish in the study of human aging and aging mitigation strategies

Aging humans experience a decline in aerobic capacity and a corresponding decline in physical ability (Fitzgerald et al., 1997; Wilson and Tanaka, 2000). Swimming performance tests can investigate the aerobic capacity of zebrafish. By recording dissolved oxygen uptake in the swim tunnel, zebrafish oxygen consumption can be measured across a range of swimming speeds (Brett, 1964; Palstra et al., 2010). Paired with cardiorespiratory and cellular respiration studies, this technique allows for zebrafish to be used as models in the study of the age-related decline in aerobic capacity.

We have tested the effect of relatively short exhaustive bouts of intermittent exercise on the swimming abilities of different ages of zebrafish. Even in similarly aged fish, different training regimes can yield different changes in muscle structure, metabolic physiology and swimming performance (Davison, 1997; Palstra and Planas, 2011). Continuous exercise at intermediate water velocities can elicit improvements in muscle mass and swimming performance and cause corresponding changes in gene and protein expression (East and Magnan, 1987; Palstra et al., 2010). Investigating the mechanisms behind the age mitigating effect of exhaustive intermittent exercise and longer, less strenuous daily training would provide a thorough basis for the development of alternative aging intervention strategies.

Further study of the relationship between sub-organism and whole-organism effects of aging and exercise could lead to innovative methods of delaying aging and mitigating age-associated diseases (Kishi, 2011). In aging humans, mitochondrial function declines in skeletal muscle, and this limits the body's ability to clear reactive oxygen species (ROS) that can build up and cause tissue damage (Short et al., 2005; St-Pierre et al., 2006; Tosato et al., 2007). Aging zebrafish likely exhibit similar decreases in mitochondrial functionality. During periods of oxidative stress, such as exercise, PGC-1α is released to stimulate expression of key mitochondrial genes associated with ROS-detoxification (St-Pierre et al., 2006). Decreased PGC-1α expression is associated with reduced mitochondrial performance in mammalian and fish aging models, making it a natural target of potential aging treatments. Exercise training-increased PGC-1α mRNA expression is known to occur in zebrafish (LeMoine et al., 2010). This increase suggests that exercise may induce defenses against sub-organismal aging. Zebrafish swimming performance and behavior could be used in high-throughput screens for effective combinations of pharmaceuticals and exercise regimes in aging intervention.

4.7. Implications for using swimming performance in future research

Researchers could account for the effect of aging on swimming performance in their studies. For example, zebrafish were used as a model for the study of amyotrophic lateral sclerosis (Ramesh et al., 2010). Swimming performance was compared across a number of transgenic lines to assess the effect of various transgenes on physical ability (Ramesh et al., 2010); however, the different transgenic lines of zebrafish were also different ages and the researchers did not account for any variation that may have been caused by age. In an exercise study, zebrafish were swum over a period of 14 months to investigate changes in swimming physiology (McClelland et al., 2006). However, they did not adjust for possible variation in swimming performance due to the fish aging over the course of the study. In sum, researchers could incorporate fish age and aging into their swimming performance models.

5. Conclusions

As zebrafish aged, their swimming performance, routine swimming activity and trainability declined. Exercise increased swimming speeds in young and middle-aged zebrafish and TBA in all ages of zebrafish, but had no effect on TBF. The increase in TBA suggests that older zebrafish responded to training, but that another factor was limiting swimming performance improvement. The effects of age and exercise on swimming performance and behavior correspond with findings from other studies using sub-organismal endpoints. Furthermore, our results are similar to findings for humans and other mammalian aging models. Zebrafish swimming performance and behavior are whole-organism endpoints that capture the consequences of aging and exercise training, warranting their use in future aging studies.

Conflict of interest

The authors have no conflicts of interests.

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